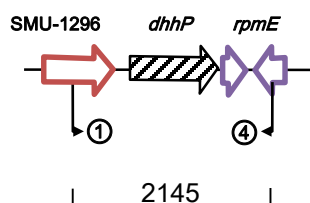
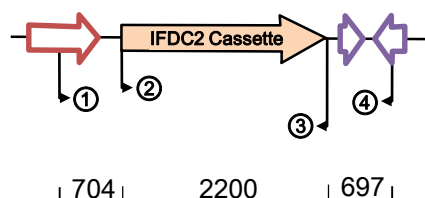
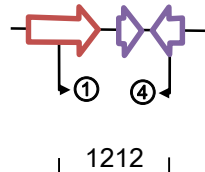
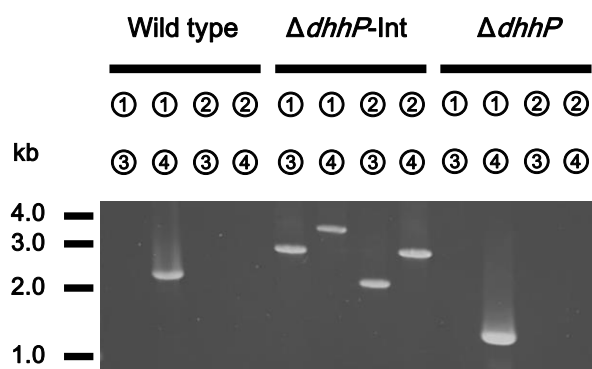


Supplemental Figure S1. Construction of a markerless mutant of *S. mutans* XC lacking *gdpP*. Chromosomal gene arrangement (A) and verifying PCR analyses (B) are shown. The *gdpP* gene was initially replaced by the IFDC2 cassette of pKOgdpP-Int, yielding an intermediate mutant strain, $\Delta gdpP$ -Int. The resulting strain was selected using erythromycin. Subsequently, the IFDC2 cassette was replaced by a linear construct containing two linked homologous fragments without the selection cassette in pKOgdpP. The transformants were then selected on plates containing *p*-Cl-Phe. The resulting markerless mutant ($\Delta gdpP$) is sensitive to erythromycin and resistant to *p*-Cl-Phe. Each circled number indicates a PCR primer used for mutant verification. The distances between primers are given in bp. Each DNA fragment was PCR-amplified using the indicated primers. DNA size standards are shown.

(A)

500 bp

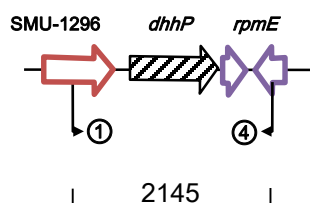
Wild type (Em^s , p -Cl-Phe r) **$\Delta dhhP$ -Int (Em^r , p -Cl-Phe s)** **$\Delta dhhP$ (Em^s , p -Cl-Phe r)****(B)**

Supplemental Figure S2. Construction of a markerless mutant *S. mutans* XC lacking *dhhP*. Chromosomal gene arrangement **(A)** and verifying PCR analyses **(B)** are shown. The *dhhP* gene was initially replaced by the IFDC2 cassette of pKOdhhP-Int, yielding an intermediate mutant strain, $\Delta dhhP$ -Int. The resulting strain was selected using erythromycin. Subsequently, the IFDC2 cassette was replaced by a linear construct containing two linked homologous fragments without the selection cassette in pKOdhhP. The transformants were then selected on plates containing p -Cl-Phe. The resulting markerless mutant ($\Delta dhhP$) is sensitive to erythromycin and resistant to p -Cl-Phe. The distances between primers are given in bp. Each circled number indicates a PCR primer used for verification. Each DNA fragment was PCR-amplified using the indicated primers. DNA size standards are shown.

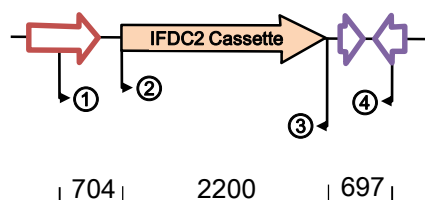
(A)

500 bp

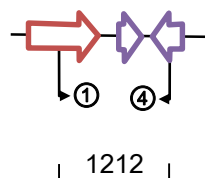
$\Delta gdpP$ (Em^s , p -Cl-Phe^r)



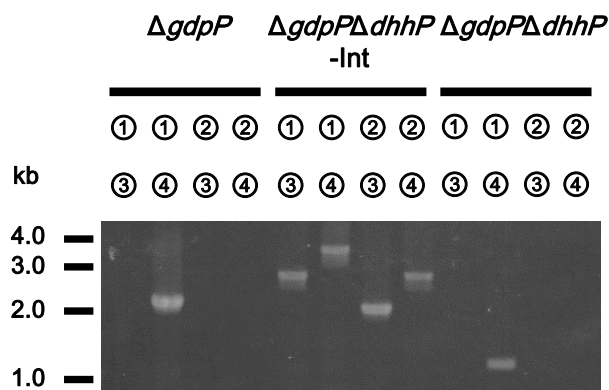
$\Delta gdpP\Delta dhhP$ -Int (Em^r , p -Cl-Phe^s)



$\Delta gdpP\Delta dhhP$ (Em^s , p -Cl-Phe^r)



(B)



Supplemental Figure S3. Construction of a markerless double mutant *S. mutans* XC lacking both *gdpP* and *dhhP*. Chromosomal gene arrangement (A) and verifying PCR analyses (B) are shown. The *dhhP* gene in $\Delta gdpP$ was initially replaced by the IFDC2 cassette of pKODhhP-Int, yielding an intermediate mutant strain, $\Delta gdpP\Delta dhhP$ -Int. The resulting strain was selected using erythromycin. Subsequently, the IFDC2 cassette was replaced by a linear construct containing two linked homologous fragments without the selection cassette in pKODhhP. The transformants were then selected on plates containing *p*-Cl-Phe. The resulting markerless mutant ($\Delta gdpP\Delta dhhP$) is sensitive to erythromycin and resistant to *p*-Cl-Phe. The distances between primers are given in bp. Each circled number indicates a PCR primer used for verification. Each DNA fragment was PCR-amplified using the indicated primers. DNA size standards are shown.